A STUDY OF THE LEAF-ANATOMY OF SOME NATIVE SPECIES OF THE GENUS *ANDROPOGON.*

[N.O. GRAMINEÆ.]

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(Plates xxix.-xxxiii.)

The microscopic anatomical examination of the leaves of some native species of the genus *Andropogon*, as discussed in the following pages, was made for two purposes. Firstly, to investigate any similarities or differences that may exist in the general and detailed internal structure of the leaves of different species; and, secondly, to investigate any œcological characteristics in the structure of the leaf, which may be associated with, and accompany, the general habit and habitat of the plant in question.

The method adopted, in preparing the sections, was briefly as follows. The fresh leaves, in small pieces, were fixed in picro-acetic acid. Great difficulty was at first experienced in eliminating the siliceous elements from the leaves; but, eventually, a successful method was found in soaking them, for some considerable time, ranging from 8 to 12 hours, in a 50 per cent. solution of hydrofluoric acid and water.

The embedding, cutting, staining, and mounting were performed by Mr. W. A. Birmingham, of the Biological Branch of the Department of Agriculture, to whom I am much indebted for the really excellent manner in which he prepared the specimens. My thanks are also due to Mr. G. P. Darnell Smith, B.Sc., F.I.C., F.C.S., Biologist, for his courtesy, and for permission to use the biological apparatus. The stains used were hæmatoxylin and erythrosin. It was found necessary to decolourise after staining in hæmatoxylin. These two stains appear to give a fairly good differentiation. The methods adopted, in embedding and mounting, were similar to those used in ordinary microtechnique.

ANDROPOGON INTERMEDIUS R.Br. (Plate xxx.).

In the widest portion of the leaf, there are nine primary bundles, *i.e.*, bundles in which the sclerenchyma is in direct contact with the phloem or xylem, or separated from the latter by thin-walled cells only. The primary bundle of the midrib is the largest. There are 33 secondary bundles, *i.e.*, bundles in which phloem and xylem are completely surrounded by chlorophyll-bearing cells. There are also present six bundles of an intermediate type. In these bundles, the sclerenchyma is developed under the bundle, but separated from the phloem and xylem of the bundle. The primary bundles are surrounded by thick-walled cells—the mestome sheath. On the outer side of these are thin-walled parenchyma-cells which, in this case, are very indistinct.

Midrib.—In the widest portion of the midrib there are three primary bundles, and these are open above and below. The chlorophyll-bearing parenchyma-cells, surrounding the middle bundle, are smaller than those surrounding the mestome bundles. The sclerenchyma on the lower surface of the midrib is well developed, but that on the upper surface consists of but few cells, which are in direct contact with the epidermal cells. The sclerenchyma on the superior surface of the other two primary bundles is much better developed than that of the middle primary bundle. The phloem tissue is extremely well developed in all the primary bundles, and this also applies to the xylem tissue. Xylem and phloem tissue are also developed in the secondary bundles, the former preponderating.

The epidermal cells on the lower surface are extremely irregular in size. The cuticle, by which they are protected, is not particularly thick, except over the primary bundles, and at the edges of the leaf. The epidermal cells on the upper surface are as small as those on the lower surface only under the secondary bundles. In all other cases, they are considerably extended in size, penetrating deeply into the mesophyll-tissue. These cells, termed by Warming hinge-cells, and known in American literature as bulliform cells, are not fan-shaped, but in rows ranging in series from 5 to 8, and situated between the primary bundles. Stomata are found on both surfaces of the leaf, the proportion on the lower surface to that on the upper surface being approximately as 3 : 1. They appear on the upper surface at the edges of the series of bulliform cells, and also occasionally about the centre of the series.

ANDROPOGON AFFINIS R.Br. (Plate xxix., fig.2).

In the widest portion of the leaf, there are eight primary bundles, forty-four secondary bundles, and three intermediate bundles.

In the midrib proper, there is only one primary bundle. On each edge of the midrib, there is also a primary bundle, and that of the midrib is not as large as the others. The chlorophyll-bearing cells of the midprimary bundle are irregular in size, and smaller than those of the other bundles. As in *A. intermedius*, they are not continued right round the bundle, but are interrupted, on both the superior and inferior surfaces, by sclerenchyma. The chlorophyll-bearing cells of the secondary bundles are much larger than those of the primary bundles, and the mesophyll-sheath is very distinct. The sclerenchyma on the inferior surface of the primary bundle is not as well developed as in *A. intermedius*, nor is that on the superior surface. The sclerenchyma on the superior surface of the primary bundles at the edge of the midrib is better developed than in any of the other primary bundles, and is in direct contact with the bundles. Generally speaking, there is not the same development of sclerenchyma right throughout the leaf as in *A. intermedius*.

The epidermal cells on the inferior surface are irregular in size, and are protected by a thin cuticle, which, however, is not nearly so well developed as that of *A. intermedius*. The epidermal cells on the superior surface are bulliform in character, with the exception of those over the primary bundles. They penetrate the mesophyll-tissue more than do those of *A. intermedius*, are distinctly fan-shaped in character, and arranged in series of 5 to

7. The stomata are present on both the inferior and superior surfaces, and in about the same proportion, approximately 3 : 1. Occasional trichomes can be seen on the inferior surface.

ANDROPOGON SERICEUS R.Br. (Plate xxix., fig.1).

Leaves of this grass, from two widely divergent localities, viz., Nyngan and the Botanic Gardens, were examined, but no essential differences between them could be found. In the widest portion of the leaf, there are five primary bundles, thirty-two secondary bundles, two intermediate bundles, and ten bundles of a secondary type, but which have sclerenchyma developed on the inferior surface.

As in *A. affinis* and *A. intermedius*, there is only one primary bundle in the midrib proper. The sclerenchyma, both on the superior and inferior surfaces of this bundle, is much better developed than is that of *A. affinis*. The development of sclerenchyma throughout the leaf, is much greater than in *A. affinis*, but not so great as in *A. ischæmum*.

The epidermal cells on the inferior surface are very irregular in character, and very many are papilliform. The epidermal cells on the superior surface partake of the character of bulliform cells except, as in *A. affinis*, above the primary bundles. They are more numerous, however, than in *A. affinis*, in series of 7 to 9, and decidedly fan-shaped in character. Trichomes and stomata are numerous on the inferior surface, and a few of the latter may be seen on the superior surface. Those on the inferior surface are, as a rule, guarded by papilla-like cells. The cuticle is comparatively thin, and, unlike *A. ischæmum*, of uniform consistency throughout.

ANDROPOGON ISCHÆMUM Linn. (Plate xxxi.).

In a transverse section, there may be seen four types of bundles, namely, primary, secondary, intermediate, and a fourth type, which has sclerenchyma developed on both the superior and inferior surfaces, but not in direct contact with it. In the widest portion of the leaf, there are eleven primary bundles, forty-six secondary bundles, twenty of an intermediate type, and two of the fourth type.

There are three primary bundles in the midrib. The middle primary bundle has sclerenchyma well developed, on both the superior and inferior surfaces. The middle primary bundle is not larger than the other two primary bundles of the midrib. Each lateral primary bundle has sclerenchyma better developed than in most of the other species. A characteristic feature of this grass is the extreme development of the sclerenchyma on the superior surface of the midrib, extending the greater part of the distance between the primary bundles. Generally speaking, the secondary and intermediate bundles of this species are more crowded and more numerous than those of the other species.

The epidermal cells on the inferior surface are very irregular in size, some being exceptionally large. The cuticle is much thicker than in *A. affinis*, *A. sericeus*, and *A. intermedius*, and is more developed under the midrib and at the leaf-edges. The cells on the superior surface are mostly bulliform in character. They differ, however, from the bulliform cells of the species previously cited, in being much wider, not fan-shaped, and protected by a well developed cuticle. Stomata are very numerous on the inferior surface, but are not as plentiful on the superior surface as in *A. affinis*, *A. intermedius*, and *A. sericeus*. Trichomes are numerous.

ANDROPOGON REFRACTUS R.Br. (Plate xxxii.).

In a transverse section of the widest portion of the leaf, there may be seen nine primary bundles. The secondary bundles are not so numerous as those cited in the other species, being only twenty in number. The intermediate bundles are very numerous, there being twenty-one of these.

The midrib is very wide, embracing bodily three primary bundles, and extending so that two other primary bundles border it at the edges. The middle primary bundle has well developed sclerenchyma on the inferior surface, somewhat wedge-shaped in character, and projecting well beyond the level of the epidermis. It also encircles the greater part of the phloem-tissue. On the superior surface, it extends from the epidermis to the xylem-tissue, and surrounds the latter throughout the greater part of its extent. The extreme development of sclerenchymatous tissue results in the elimination of many of the chlorophyll-bearing cells, so that the latter are only few in number.

All the primary bundles have the sclerenchyma well developed, and in direct contact with the phloem- and xylem-tissue. It does not, however, surround the phloem- and xylem-tissue as in the case of the primary bundle; and, consequently, the chlorophyll-bearing cells are more numerous and more regular in size than in the case of that bundle. The intermediate bundles are completely surrounded by chlorophyll-bearing parenchyma, but the sclerenchyma is extremely well developed on the inferior surface, and projects well beyond the level of the epidermis.

The epidermal cells on the inferior surface are uniform in size. Over many of the primary and intermediate bundles, they become more or less lignified, and take on the character of sclerenchyma-cells. Between the masses of sclerenchyma mentioned above, the epidermal cells are arranged in grooves. In some cases, the apical cell of the conical masses of projecting sclerenchyma is extremely large. Throughout the whole of the inferior surface, the epidermal cells are protected by an extremely thin cuticle. The epidermal cells on the superior surface take on the character of bulliform cells. These are noteworthy inasmuch as they form a double row of cells, the bottom row being much larger than that above. These cells extend throughout the whole space between the primary bundles, and have not the fan-shaped characteristics present in the other species. The stomata are numerous on the inferior face, and, in most cases, are sunk in the epidermal grooves between the masses of sclerenchymatous tissue. A few stomata may be seen on the superior face, but they are not as plentiful as in the other species.

ANDROPOGON BOMBYCINUS R.Br. (Plate xxxiii.).

The anatomical structure of a leaf closely resembles that of *A. refractus*. The principal points of difference are : —

(1.) The number of secondary bundles is smaller.

(2.) The sclerenchymatous masses developed under the primary and intermediate bundles are larger.

(3.) The epidermal cells on the superior surface have subimposed two, three, and sometimes four layers of similar uncoloured parenchymatous cells.

(4.) No stomata can be perceived on the superior surface.

(5.) A girdle-canal is present.

From an anatomical standpoint, the species under discussion may be divided into three groups, viz.:—

Group i. *Andropogon intermedius.*
A. affinis.
A. sericeus.
Group ii. *Andropogon ischæmum.*
Group iii. *Andropogon refractus.*
A. intermedius.

In considering the first group, it may be seen that there are certain well defined points of difference between *A. intermedius* and the other two species; and also less defined differences between *A. affinis* and *A. sericeus.* In *A. intermedius*, there are more primary bundles in the midrib, than in *A. affinis* or *A. sericeus.* The intermediate bundles, with a corresponding development of sclerenchyma, are also more numerous than those of the other two species; and the bulliform cells are not as distinctly fan-shaped. *A. sericeus* differs from *A. affinis* only in the slightly greater development of the sclerenchyma, and in the slightly greater number of bulliform cells in each series.

A. ischæmum differs from the first group in the much greater development of the sclerenchymatous tissue, which, however, is not as great as that of the third group. The cuticle is also much thicker, on both the superior and inferior surfaces, than that of the previous species. Another characteristic difference is the nature of the vascular bundles, which are much more numerous, and more densely crowded than in any of the other species.

A. refractus and *A. bombycinus* differ widely from any of the species of the other two groups. The extreme development of sclerenchymatous tissue, the thick nature of the cuticle on both the superior and inferior surfaces, and the arrangement of stomata in grooves on the inferior surface, are well marked characteristics, which are not seen in any of the other species.

Ecological characteristics.

The morphological structure of the leaves of *A. refractus* and *A bombycinus* shows well marked xerophytic characteristics. The well developed sclerenchymatous tissue, besides aiding in the mechanical strength of the leaf, also has a connection with the dry environment (*see* Warming, Ecology of Plants). The cuticle is also extremely thick on both the superior and inferior surfaces, thus depressing transpiration.

The presence of strata of water-storing cells, on the superior surface, is also a well marked xerophytic characteristic. Whether the bottom layer functions as hinge-cells, curling up the leaves on losing their turgescence, is uncertain. It has been pointed out by Warming and others, that, when hinge-cells occur and function in rolling the leaf when diminished transpiration is necessary, the stomata mainly occur on the superior surface, and are thus protected. The stomata, in the two species in question, are mostly confined to the inferior surface; and it is difficult to see what advantage the leaf would have, so far as the stomata are concerned, if it were rolled up. The stomata, being arranged mostly in grooves, are well protected, when the leaf is in the flat position. This xerophytic stomatal arrangement is also referred to by T. Holm* in discussing some of the lowland species of the Rocky Mountains, Colorado,

The presence of "girdle-canals" in *A. bombycinus* is also probably a xerophytic characteristic, designed to depress transpiration. These are referred to by Giltay, as occurring in some arenicolous grasses.

The habitat of these two species corresponds, to a very large extent, with their xerophytic characteristics. *A. refractus* is distributed over a great part of New South Wales, and is commonly foumd in sandy and rocky situations *A. bombycinus* is confined mostly to the interior, and is often found on sand-hills.

In *A. ischœmum*, the xerophytic characteristics are not as well marked as are those of *A. refractus* or *A. bombycinus*, but it may be considered more xerophytic than *A. intermedius*, *A. affinis*, or *A. sericeus.* This is seen in the crowded vascular bundles, the amount of sclerenchymatous tissue, and in the thick cuticle. Although the bulliform or hinge-cells on the upper surface are distinctly fan-shaped, the advantage in the folding of the leaf is somewhat uncertain, as most of the stomata are confined to the inferior surface.

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* Botanical Gazette, Vol. xlvi.

A. ischæmum is a grass well adapted to sandy and rocky situations. In "L'Herb. Bossier" (vi., 971), it is mentioned as occurring commonly in sandy places in the Geneva Valley; and also in rocky places on the side of the Alps. In Australia, it grows on the Drummond Ranges in West Australia

A. intermedius, *A. affinis*, and *A. sericeus* do not show, in their structure, any well marked xerophytic characteristics, and are inclined to be more mesophytic in their nature. This is seen in the comparatively small amount of sclerenchymatous tissue, the comparatively thin cuticle, and in the distribution of the stomata, which are numerous on both faces of the leaf.

A. intermedius is spoken of as thriving best on river-banks in New South Wales. In Africa, a variety of this grass is also mentioned by Stapf as commonly occurring on the banks of rivers.

A. sericeus, however, is commonly spoken of as a drought-resistant grass; but I cannot reconcile this view with my practical experience with it. Rather does its habit correspond more to the mesophytic type. The grass has died out at Cowra, Wagga, and Bathurst Experiment Farms; and although a good catch was secured, under moist conditions, at Nyngan Demonstration Farm, the plot is always quick to show the effect of dry weather. On the other hand, the structure of the leaf, with its numerous stomata and comparatively thin cuticle, indicates the possible adaptability of the grass to irrigable conditions. This has been practically demonstrated at Bathurst Experiment Farm, where the grass made a profuse and succulent growth under irrigation. As soon as the effects of the latter were removed, the grass quickly died out.

My experience has also been that, in its natural state, it is more commonly found on the better and moister soils; and that, even in the plains of the interior, although it responds readily to rain, it will quickly "brown-up" under dry weather-conditions.

EXPLANATION OF PLATES XXIX.-XXXIII.

Explanation of References.

S, Stereome—P, Phloem—X, Xylem—C.P, Chlorophyll-bearing Parenchyma—B, Bulliform cells—M.S, Mestome-sheath- St, Stoma—U.P, Uncoloured Parenchyma—$M.B_1$, Primary Mestome-sheath—$M.B_2$, Special form of secondary bundle—$M.B_3$, Secondary Mestome-bundle—$M.B_4$, Intermediate Mestome-bundle.

Transverse sections of leaves of species of *Andropogon.*

Plate xxix.

Fig. 1.—*Andropogon sericeus* R. Br.
Fig. 2.—*A. affinis* R. Br.

Plate xxx.

A. intermedius R.Br.

Plate xxxi.

A. ischæmum Linn.

Plate xxxii.

A. refractus R.Br.

Plate xxxiii.

A. bombycinus R.Br.